Title: **Site-Specific Assessments of Environmental Risk and Natural Resource Damage based on Great Horned Owls**

Authors: **Matthew J. Zwiernik,[*†] Karl D. Strause*, Denise P. Kay,[‡] Cyrus S. Park,[†] Alan L. Blankenship,[†‡] and John P. Giesy,[†‡§]**

[†]Zoology Department, Center for Integrative Toxicology, National Food Safety and Toxicology Center, Michigan State University, E. Lansing, MI 48824

[‡]ENTRIX, Inc., Okemos, MI 48864

[§]Dept. Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan, Canada and Biology and Chemistry Department, City University of Hong Kong, Kowloon, Hong Kong, SAR, China.

* Correspondence may be directed (Co-senior authors)

Dr. Matthew Zwiernik
224 National Food Safety ad Toxicology Bld.
East Lansing, MI 48848
zwiernik@msu.edu
Ph: 517-749-5243
Fax: 517-432-2310

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ABSTRACT

Selection of receptors is a key element of effective risk and natural resource damage assessments. This is especially critical when site-specific field studies are employed. The great horned owl has advantages over other species as a key tertiary terrestrial receptor that can be used as an integrated measure of exposure to residues in a multiple lines of evidence approach. The methods described herein, allows for minimization of uncertainty in assessment endpoints, while also minimizing the potential impact of the study on populations and maximizing the utility of data in testing of hypotheses. These methods exploit attributes of the great horned owl (*Bubo virginianus*), including its propensity to nest in artificial nesting platforms, which allows for better control of experimental conditions than normally experienced in studies of wildlife. The data collected are supportive of a multiple lines of evidence approach including the elucidation of contaminant exposure by both predicted (dietary) and tissue-based methodologies. In addition population-level measures of potential effects including productivity and abundance can be directly measured. Over the course of 5 yrs, 48 artificial and 6 natural great horned owl nests, covering approximately 14 active territories along approximately 38 km of river floodplain, were monitored for activity at the Kalamazoo River Superfund Site in Kalamazoo and Allegan Counties, Michigan. There were 25 nesting attempts observed in 20 active nests. Residue concentrations of polychlorinated biphenyls (PCBs) and *otho*- and *para*-substituted isomers of DDT, DDD and DDE (SDDTs) were measured in 24 eggs and 16 samples of nestling blood plasma. Exposure through the diet was predicted by determining a site-specific dietary composition (based on 285 dietary items) followed by sampling and quantifying residue

(PCBs) concentrations in 171 identified prey items that were collected from the locations where owls had taken the prey. Hazard assessments based on measured concentrations in tissues and based also on predicted concentrations in the diet produced similar results that indicated minimal risk to resident GHO populations (Hazard Quotients $\leq$ 1.5). The number of GHO present in an area was highly correlated with the number of attempted breeding events. The use of convergent lines of evidence resulted in greater confidence in the assessments of both exposure and potential effects. Repeated use of artificial nesting platforms by GHOs minimized temporal and spatial variability. The GHO was found to be a useful receptor for evaluating terrestrial contaminant exposures and associated risk utilizing a multiple lines of evidence approach.

Keywords: ERA, receptor, raptor, great horned owl, exposure assessment, multiple lines of evidence

INTRODUCTION

Raptor species have long been used as environmental monitors (I.J.C., 1991; Sundlof, S.F. *et al*., 1986; C.E.Q., 1972) because they are sensitive to some of the more frequently observed prevalent contaminants of concern (COCs), and have a high potential for exposure to those residues. Herein we describe direct, site-specific, field assessment methodologies, that use the great horned owl (*Bubo virginianus;* GHO) as a sentinel or surrogate species for terrestrial-based organisms in assessing ecological hazards or natural resource damages as well as site-specific clean-up values for soils. The methodologies take advantage of useful attributes of the GHO in a multiple-lines-of-evidence approach to assess potential exposure to COCs and potential subsequent effects. Exposure was quantified both by predicting exposure through the diet and by measuring concentrations in blood plasma and eggs of GHO. Both estimates of exposure were then compared to threshold concentrations for effects reported in the literature. Measures of abundance and reproductive performance were used to confirm the predicted magnitude of effects. The methods were designed to minimize uncertainty in assessment endpoints (Fairbrother, 2003), minimize the ecological impact of data collection, and maximize the utility of data in testing hypotheses.

SPECIES APPLICABILITY

Guidelines promulgated by the United States Environmental Protection Agency (US EPA) state that species-specific as well as site-specific factors dictate the applicability of an organism for use as a species of concern in risk assessments performed under the "Comprehensive Environmental Response, Compensation, and Liability Act" (CERCLA) based ecological field studies (USEPA, 1994, 1997, 1999). The ultimate goal is to select specific populations or communities for which the collected data and resulting decisions can be extrapolated across the ecosystem of interest. Both the GHO and the specific methods described herein, have a broad applicability to key ecological components.

Comparisons of measurement endpoints for GHOs can be made across wide geographical regions and habitat types. The GHO is endemic throughout the temperate and sub-arctic regions of the Americas from Alaska to Argentina and has one of the largest ranges of all raptors (Houston, C.S. *et al*., 1998; Burton, J.A., 1984; A.O.U, 1983). In addition, it is able to utilize more habitat types than any other American raptor species (Johnsgard, P.A., 1988) while maintaining a foraging range and taxonomic dietary composition that is similar to a number of less adaptive medium and large terrestrial-based receptors (Austing and Holt, 1966; Austing, G.R., 1964; Craighead and Craighead, 1956).

In addition to geographic applicability, a number of species-specific characteristics need to be considered when selecting organisms for study. These include intensity (concentration) and duration (time spent on-site) of exposure, appropriateness as a surrogate species, sensitivity to some of the primary contaminants of concern at many sites, including the Kalamazoo River Superfund Site (KRSS), ecological function, relative ease of conducting field studies with the organism, and other recognized values (USEPA, 1994). The GHO is a top food web predator and year round resident throughout its range. GHOs are strict carnivores with large rates of ingestion, relative to their body weight (Tabaka, C.S *et al*., 1996) and have life spans known to exceed 28 yr (Nero, R.W. 1992). These attributes, as well as the fact that GHOs have no known predators, makes the GHO a useful indicator of the magnitude and bioavailable fraction of contaminants in terrestrial ecosystems.

GHOs are considered to be among the most sensitive animals to some of the most common environmental contaminants that occur in terrestrial environments (Hoffman, D.J., 1995). Dietary exposure of owls to small amounts of select contaminants such as organophosphates (OP), organochlorines (OC) and metals has been shown to cause lethal and sub-lethal effects including reproductive impairment or failure (Sheffield, S.R., 1997). Because of these characteristics, the GHO is a useful sentinel or surrogate for other terrestrial species, or as a bio-indicator or bio-monitor for evaluating potential exposures of avian populations to contaminants (Sheffield, S.R., 1997).

The nesting characteristics of GHOs provide advantages as indicators of contaminant bioavailability relative to raptors. In terms of both geographical location and habitat diversity, the GHO occupies the greatest range of nesting sites of any bird in the Americas (Baumgartner, F.M., 1938). GHOs do not construct their own nests, but rather commandeer the nests of others, which are typically nests of squirrels, red-tailed hawks or crows. For this reason, GHOs will utilize artificial nesting platforms (Bohn, R.T., 1985; Holt, J.B., 1996). GHOs will continue to use a nest as long as it remains successful and serviceable. GHOs do not maintain their nest. Natural nests, especially usurped nests, are rarely used for more than a single season (Frank, R.A., 1997; Holt, J.B., 1996). As a result, GHOs are almost always looking for a new nest within their territory and quickly move to constructed nesting platforms. The use of artificial nesting platforms obviates the need to locate and access natural nests and simplifies monitoring of GHOs. In addition, platforms allow for the dictation of foraging areas, consistency among years, and minimization of predation. Constructed platforms can be durable, placed in a wide range of locations, and maintained indefinitely. The resulting multi-year use of the same nest reduces variability in temporal and spatial exposure profiles.

GHOs offer advantages over other tertiary terrestrial receptors when assessing site-specific COC exposures and population health. As top predators, GHOs effectively integrate exposures to COCs from multiple trophic levels and habitats. Like most higher order, terrestrial predators, GHOs are opportunistic feeders with a diet that includes a wide variety of small- and medium-sized mammals, birds, insects, and amphibians (Marti and Kochert, 1996; Voous, K.H., 1988; Marti, C., 1974; Craighead and Craighead, 1956). Exposures of GHO nestlings to residues have been shown to be directly related to local contaminant concentrations (Frank, R.A., 1997) and their abundance has been shown to be directly related to available prey (Rohner C., 1996; Houston and Francis, 1995; Rusch, *et al*., 1972; Adamcik, *et al*., 1978) and ultimately ecosystem health.

Concentrations of COCs in GHO can be directly assessed through the collection of tissues, eggs, or blood. GHOs have relatively great rates of reproduction, a factor that offers advantages in meeting sample size requirements. GHOs are relatively easy to capture and handle as compared to other terrestrial-based raptors. Nestlings between 5 and 6 wks of age can be easily accessed, banded, morphological characteristics measured, blood sampled, and radio tagged (Austing and Holt, 1966). Broods of pre-fledge nestlings (typically one to three individuals) are confined to the nest and rely solely on prey collected by adults from areas proximal to the nest. Parental foraging ranges of GHO decrease during rearing due to nest defense and prey transport limitations. This ensures that exposures of both adult and nestling GHOs to COCs are directly linked to the immediate area surrounding the nest site.

Exposure of GHOs through the diet can be quantified by enumerating the composition of the diet and determining the concentrations of COCs in the prey items. These two parameters can then be combined to allow calculation of a weighted average concentration of COCs in the diet and an average potential daily intake (USEPA, 1993). Methods to determine site-specific dietary composition are well described and include the combined examination of prey remains and regurgitated pellets (Marti, C.D., 1987; Rusch, D.H., *et al*., 1972; Errington, P.L., 1930). Unlike other raptors, owls prefer to swallow their smaller prey items whole. The prey enters the glandular stomach where enzymes break it down. Undigested materials, such as bones and hair are regurgitated in the form of a packed pellet within 2 to 24 h after consumption. These pellets along with prey remains in and around the nest can be sampled over time.

THE GHO AS A KEY RECEPTOR (CASE STUDY)

The studies and results reported here were part of a large group of studies in support of an ecological risk assessment of the KRSS (Blankenship, A.L., *et al*., 2005; Kay, D.P., *et al*., 2005; Millsap, S.P., *et al*. 2004; Neigh, A., *et al*. 2006a,b; Strause, K.D., 2006). The Kalamazoo River Area of Concern (KRAOC) was designated a Superfund site in 1990 and is comprised of nearly 100 Km of the Kalamazoo River from Portage Creek in the city of Kalamazoo to the downstream terminus at Lake Michigan. The primary COCs were identified as polychlorinated biphenyls (PCBs) with some evidence of elevated exposures of raptors to DDT and its metabolites DDE and DDD (hereafter, SDDT) (Mehne, C., 1993).

The GHO study was designed to determine bioavailability and accumulation of the COCs from a terrestrial food web in the contaminated floodplain of the Kalamazoo River. The potential for adverse effects to resident GHO populations was estimated using a hazard quotient (HQ) approach which was validated by comparisons to the abundances and reproductive productivity of GHOs in the target study areas, as well as reference areas, and information available in the literature about these population parameters at other uncontaminated locations.

METHODS

Study site

The study area included sections of the Kalamazoo River, both upstream and downstream of known sources of contaminants. Four contiguous study areas of ≥7km were utilized including two target areas, Lake Allegan State Game Area and the former Trowbridge impoundment, as well as two upstream reference sites at Fort Custer State Recreation Area and Ceresco Impoundment (Figure 1). Upstream or reference locations were selected based on habitat suitability and applicability to baseline watershed contaminant exposures, and included two areas encompassing 15 km of free flowing and impounded areas of the river. Floodplain habitats included emergent marsh, wet meadow, emergent shrub and deciduous forested wetland. For the downstream and contaminated target areas, study locations included similar habitats of free flowing, impounded, and formally impounded sections of the river. Specific areas were selected based on a maximum

Fig. 1

potential for exposure of resident owls to the COCs from floodplain soils during foraging activities associated with nesting and subsequent rearing of offspring (Strause, K., 2006).

Artificial nesting platforms

Nest platforms were constructed, with minor modifications, as described in Henderson [1992]. In brief, a 3.5’ x 3.5’ piece of 1 in “chicken wire” mesh was cut into a circle and formed into a nesting cone by making one cut from the outer edge to the center and then overlapping the two cut edges until the cone is about 18 in deep. The cut ends of the chicken wire were bent around the overlapping ends to hold the cone together and to prevent sharp ends from protruding. A 3.5’ x 3.5’ section of dark gray Tyvek® was similarly cut, folded and placed into the wire cone. Tyvek® is strong, lightweight, and breathable and provides protection from weather, light, and moisture. A drainage hole was cut at the base of the Tyvek® cone and leaf litter was placed between the wire mesh and Tyvek®. Flexible 1/2” and smaller stems of willow and dogwood were woven, placed at the top edge, and spiraled around the inside of the nest working down. Stems were secured with light gauge stainless steel wire by wrapping the wire around the branches and through the wire frame and twist-tied on the outside of the nest (Figure 2). Once installed one or two liters of shredded wood chips were added to the inside of the cone as additional nesting material. Fig. 2

Placement of nest platforms

Nest platforms were placed in live trees of at least 25 cm in diameter at the base. Preferred sites included large trees on the leeward edge of shelterbelts or other areas somewhat protected from high winds. Effective nest placement was no less than 8 m from the ground and ideally at 11.5 to 16.5 m. Pre-constructed nests were secured in a suitable crotch with camouflaged stainless steel adjustable pipe clamps. Because exposure to PCB contaminated floodplain soils was being evaluated, nests were located within 100 m of preferred foraging habitat and offered GHOs a combination of concealment, easy flight access, and proximity to selected foraging grounds. Ten to fifteen nests were deployed per study area resulting in a density of 1-3 artificial nesting platforms per breeding territory. In all, a total of 54 nests were monitored including six natural nests.

Exposure based on measured concentrations

The first measure of exposure included concentrations of PCBs and SDDT in eggs and nestling blood plasma. Eggs were collected as soon as incubation activity was confirmed. Individual eggs were placed in pre-labeled, shock-absorbing, crush-proof transport containers placed in a pack and carried to the ground. Eggs were labeled, transported back to the laboratory and stored at 4 ºC until processing. Weight, volume and eggshell thickness of eggs were determined (Stickel, J., et al., 1973). The contents of the eggs were then homogenized. Blood was collected from nestlings by use of sterile technique (Frank, R.A., 1997; Henny and Meeker, 1981; Buck, J.A., et al., 1996) when they were 5 to 6 wk of age and greater than 0.75 kg . Owlets at this stage were relatively easy to capture, tolerated handling and could be returned unharmed to the nest. Blood was drawn with 26-gauge hypodermic needles into 10 ml syringes containing sodium heparin solution and then transferred to pre-labeled heparinized Vacutainers™ and placed on cold packs in an insulated cooler. During the collection of nestling blood samples, individual nestlings were identified by attaching leg bands (United States Fish and Wildlife Service (USFWS) #9 rivet) following standard USFWS protocols. Addled eggs and egg shell fragments also were collected at the time of banding. Vacutainers™ containing whole blood were transported to the laboratory and centrifuged at 1200 rpm for 10 min and the plasma (supernatant) was transferred into a new green top Vacutainer™ appropriately labeled and stored upright at –20 ^{o}C.

Quantification of select COCs was performed at the Michigan State University (MSU) Aquatic Toxicology Laboratory (ATL) based on project-specific data quality objectives. Total concentrations of PCBs(congener-specific analysis) and SDDT were determined using EPA method 3540 (SW846), soxhlet extraction, as described elsewhere (Neigh, A., et al., 2006b). Briefly, concentrations of PCBs, including di- and mono-*ortho*-substituted congeners were completed by gas chromatography equipped with a ^{63}Ni electron capture detector (GC-ECD). Concentrations of non-*ortho*-substituted PCB congeners and SDDT were determined by gas chromatograph mass selective detector (GC-MS). The limit of quantification (LOQ) for di- and mono-ortho-substituted PCBs was conservatively estimated to be 1.0 ng PCB/g, ww. For coplanar PCB congeners and SDDT analytes, method detection limits (MDLs) varied among samples but were maintained for all samples at <0.1 ng/g, ww. Either TurboChrom (Perkin Elmer, Wellesley, MA, USA) or GC Chemstation software (Agilent Technologies, Wilmington, DE, USA) was used to identify and integrate the individual PCB congener peaks. Total concentrations of PCBs were calculated as the sum of all resolved PCB congeners.

Dietary Exposure

The site-specific exposure to PCBs via the diet was predicted by determining the relative proportions of prey items in the diet followed by measurement of PCBs in representative samples of those items collected from the reference and target floodplain study locations. Site-specific dietary composition for resident owls was determined from prey of actively nesting GHOs. Prey items included regurgitated pellets and any uneaten remains of prey such as bones, feathers, scales, and fur (hereafter referred to together as prey remains). All prey remains were collected from around the nest tree and beneath feeding perches prior to egg drop and incubation. Prey remains were again collected from within the nest, around the base of the nest tree, and below any associated feeding perches at time of banding and subsequently at 10-d intervals until sampling was no longer productive. Use of this method ensured minimal nest disturbance while insuring that fresh prey remains were being collected. The systematic and complete removal of prey remains was done to reduce the chance of overestimating the frequency of occurrence of large prey species because of their tendency to be represented in more than one pellet or prey sample (Marti, C., 1974). Prey remains were placed into containers and individually labeled as to collection time and relation to nest. Prey remains collected from within the nest were limited to those items, which were fully consumed. Partially consumed prey items were not collected and instead were noted as to species and size.

Relative proportions of prey items in the diet were determined by examining unconsumed prey remains (bones, fur and feathers of animals too large to consume whole) as well as skeletal remains in regurgitated pellets (Hayward, J.L., *et al*., 1993).

Prey items were identified down to the lowest practical taxonomic classification and grouped by species, family or order. Pellet contents were quantified as to the minimum number of individuals from each taxon necessary to account for the assemblage of remains. For prey items too large to swallow whole (> 100 g), individual time points and collection sties were examined together to reconcile the frequency of occurrence of larger prey species when remains of the same prey item were present in multiple samples. Multiple prey item identification keys were utilized for comparative identification including owl pellet identification keys (Carolina Biological Supply Company, Burlington, NC) and the vertebrate skeletal collection from the MSU museum. Avian remains were identified with the aid of MSU Kellogg Biological Station (KBS) bird sanctuary personnel. Dietary composition was based on the frequency of occurrence of all identifiable prey items and compiled on the basis of absolute (%) frequency of occurrence and relative (%) composition of biomass.

Prey item sampling

Once identified as a principal component of owl diet, prey species were collected from the most contaminated GHO foraging areas and a reference location. Species selection and sample sizes were determined based on sensitivity and power analyses of preliminary data and expected contribution to GHO dietary exposures.. For this study a total of 171 small mammals including meadow voles, white-footed mice, deer mice, meadow jumping mice, eastern chipmunks, short tail and masked shrews were sampled from six locations at two time points. Also sampled from these locations were arthropods, including four orders each of terrestrial and aquatic invertebrates. Larger mammals such as red squirrels, grey squirrels, cottontail rabbits, muskrats, and mink as well as passerine species including the American robin, house wren and tree swallows were sampled opportunistically throughout the study area. Sampling techniques varied depending on target species.

Hazard Evaluation

Here we provide methodologies for site-specific assessment of the hazard of chemicals in soils to GHOs based on a multiple lines of evidence approach that could enable well-informed decisions regarding potential remedial actions and determination of natural resource damages (EPA, 1997; Fairbrother, A., 2003). Such an approach has been applied at other contaminated sites for wildlife species such as mink (Bursian, S.J., et al., 2003) or tree swallows (Custer, C.M., et al., 2005). However, to our knowledge, this is the first case in which field studies and multiple lines of evidence have been utilized to assess potential risks of PCBs and SDDT to GHOs. The multiple lines of evidence included several methods of estimating exposure. Direct observations of population densities and reproductive success were made and compared to the results of the hazard assessment. Exposure of GHOs to these compounds was characterized in two ways. Concentrations of PCBs in the diet were calculated from the site-specific dietary composition and concentrations in prey items, as well as measured concentrations of PCBs and SDDT in eggs and blood plasma of nestling GHOs. Each measure of exposure was compared to the threshold for a toxic effect determined from the literature and expressed as a toxicity reference value (TRV) (USEPA, 1998). An ecological risk assessment (ERA) was conducted by calculating HQs. HQs were determined by dividing the measured or predicted concentration in the diet, egg or nestling blood plasma by the appropriate TRV. Comparisons were made between, within, and among, sites, individuals and prey species. The multiple lines of evidence approach can be optimized, based on information needed, level of effort available, and site-specific criteria and characteristics.

Population Density and Reproductive Success

The final line of evidence included measurements of population health. Health of the GHO population was assessed through the evaluation of productivity including, nest success, number of nestlings per nest, fledging success, and nestling age and growth measurements as well as species abundance. Much of the information on population dynamics was acquired in conjunction with the owl banding and nest monitoring tasks described above. However, an additional effort was made to evaluate GHO population health using vocalization surveys.

Vocalization surveys

The results of vocalization surveys and triangulation were used to identify active breeding territories, locations of nests, site use, relative abundance and confirmation of fledging success. A combination of vocalization survey methods were used including an active method in which GHO hoots were broadcast to provoke responses (Frank, R.A., 1997; Brenner and Karwoski, 1985), and a passive or silent survey method during sensitive lifestage events and the periods when GHOs were most active (e.g., just before and during mating) (Rohner and Doyle, 1992). Relative abundance determinations were made based on the number of individuals responding on a per survey basis. Pair vocalization responses and post survey observations were evaluated and referenced to literature-based foraging areas to delineate active territories. Nestling fledge success was determined by nestling vocalizations post banding and/or subsequent visual confirmation. All positive responses and non-responses were recorded. For the positive responses, sex and age (adult or juvenile) and global positioning system (GPS) coordinates of river location, and approximate azimuth values (compass readings) of response origin were recorded for the purpose of location by triangulation. Post surveys, targeted areas of 150 m radius were searched systematically by foot for signs of GHO activity (white wash and castings) to determine roost sites and to locate nests. Active or potentially active roost sites and nest locations were recorded using a GPS receiver. To minimize disturbance to incubating birds, ground activity during the months of February and March was limited to occupancy identification of previously located nests and known nesting platforms. Nesting activity was confirmed visually by spotting scope from predetermined monitoring locations no less than 50 meters from the nest or by overhead flights using fixed wing aircraft.

RESULTS

Over the course of five years, monitoring efforts were completed at 48 artificial and six natural nests covering approximately 14 active territories and approximately 38 km of river. Nesting activity was observed at 20 individual nests and resulted in 25 nesting attempts. Of the 20 nests utilized by GHO, five were natural nests, including four appropriated nests of other avian species and a tree cavity nest. Artificial nesting platforms were successful in attracting GHOs to preferred study area in the floodplain. In fact, nesting activity did not occur in natural nests in those territories for which artificial nesting platforms were in place. Reuse of artificial nesting platforms over multiple seasons allowed for the minimization of temporal and spatial variability and allowed easy access for researchers. The robust owl population was ideal for evaluating the multiple lines of evidence at both the study and reference sites.

Detailed methods and results for contaminant analysis and exposure assessments are provided in separate papers (Strause, K.D., *et al*., 2006a,b). The results are summarized here to illustrate the effectiveness of the methods and as an example of sample sizes that may be necessary to detect differences between the study and reference locations.

GHO Exposures were assessed by collecting both fresh eggs (destructive) and nestling blood plasma (non-destructive). Sample availability varied among years and locations (Table 1). A total of 40 egg and blood plasma samples were collected. Of the 24 eggs collected during the study, five were from the reference areas and 19 were from the target areas. Blood plasma was collected from 16 individual nestlings, this included four samples from the reference areas, and 12 samples from the target areas.

Table 1

Statistically significant differences in concentrations of total PCBs were observed among locations (reference vs. target) for the predicted dietary exposure and for total PCB and SDDT concentrations in GHO eggs and blood plasma (Strause, K.D., *et al*., 2006a,b). These differences were the result of exposures to mean PCB concentrations in floodplain soil of approximately 0.17 mg PCBs/kg, dw (dry weight) in reference areas and approximately 15 mg PCBs/kg, dw in the target areas. Differences in dietary composition between the reference and target areas also were observed (Figure 3). Differences between predicted dietary exposures (average potential daily dose) were largely the result of significant differences in concentrations in the prey items (Table 2), and were not a product of differences in dietary prey item composition. Concentrations of PCBs and SDDT in eggs were significantly different between reference and target areas (Figure 4). Diet-based HQ values calculated from geometric mean total PCB concentrations in prey animals collected from the most contaminated areas of the KRSS floodplain were less than 1.0 at both study locations. Tissue-based HQs calculated from the geometric mean concentrations of total PCBs and SDDT in eggs were ≤ 1.5 at all target locations (Strause, K.D., *et al*., 2006a). In addition, a well defined relationship was established for total concentrations of PCB in eggs and those in nestling blood plasma (Strause, K.D., *et al.*, 2006c). The statistical power of the tests were such that statistically significant differences (Type I error (α) of 0.05 and Type II error (β) < 0.20) in exposure could have been detected with as few as 4 eggs or 12 samples of nestling blood plasma per area.

Table 2

Fig. 4

Relative abundance of GHOs per river km, were significantly different between the reference and target areas of the Kalamazoo River, but reproductive productivity per defended territory (number of nestlings fledged per active nest) was not significantly different between study sites. During the three-year period (2000 – 2002) in which abundance measurements were completed at the KRSS, significant differences in the number of adult, juvenile and paired responses of GHOs were observed, with the Trowbridge impoundment (target area) having greater numbers of each response compared to Ft. Custer (reference) (Table 3). The Trowbridge impoundment had a

Table 3

greater number of active nests (6 vs 1) and greater overall recruitment to floodplain populations with six successful fledglings compared to one successful fledgling at Ft. Custer, however, the mean rates of productivity for the two sites were identical at 1.0 fledgling per active nest (Table 3).

DISCUSSION

Use of the GHO as a key receptor species in ERAs is predicated on its relatively great exposure potential, broad applicability among geographic regions and ecosystems, and ease of study. While the first two characteristics have been well documented for the GHO, its nocturnal nature and aggressive disposition may have previously dissuaded researchers from using the species in previous ERAs. For this study, the GHO proved to be a relatively easy and effective receptor species with applicability to both screening level and site-specific baseline ERAs. The single most important outcome of this study was our ability to induce breeding pairs of GHOs to occupy nesting sites centrally located within areas of interest and reuse those nesting sites over multiple years. This provided for conservative and worst case exposure assessment evaluations and risk characterizations. These behavioral attributes of the GHO offered significant advantages over other top terrestrial food web receptors including all other large resident raptors.

The strategy of conducting initial surveys to identify occupied GHO territories, followed by reconnaissance of active owl territories within the areas of interest was effective for locating existing owl territories. However, successful location of optimally located natural nests (in relation to contaminated floodplain foraging habitats) was rare. Site-specific characteristics indicate that this may have been due to an absence of available nests in the floodplain of the study area because other nest building species are more limited in nesting habitat and prefer upland areas. Artificial nesting platforms were placed inside the perimeter of defended territories and centrally located within the areas of interest. The density of nesting platform placement ranged from 1 to 3 nests per territory at 500 to 1000 m intervals. Over the five-year study period, nesting activity was identified in 81% of the territories containing nesting platforms. Nesting activity occurred in 90% of territories in which paired owls were identified. Both relative abundance and pair response were useful predictors of nesting potential. Nesting activity in natural nests was never observed in those territories in which artificial nesting platforms were placed at least 6 mo prior to expected egg drop. Platforms were utilized preferentially even in instances where appropriate natural nests were available and/or when natural nests were utilized the year prior to artificial nest placement. Breeding and reproductive success of nesting pairs utilizing artificial nesting platforms was comparable to natural nest-based reproductive studies. Of the territories in which a platform had been placed, GHOs initiated incubation 65% of the time. In a 28-yr study, in a proximal geographical area of similar characteristics, it was found that 62% of owls in occupied territories initiated incubation. For that study, the resulting annual mean productivity expressed as the number of young/occupied territory varied moderately from 0.5 to 1.1 and the number of fledglings/successful nest was a steady 1.7 (Holt, J.B., 1996). In the study on which we report here reproductive productivity in both the reference and target was similar to the Holt study. The annual mean number of juveniles fledged per occupied territory ranged from 0.5 to 1.6 and the number of fledglings/successful nest was 1.4. Post-fledge survival was successfully monitored in all territories in which systematic active surveys were performed and nestlings were banded. Monitoring of survival of juveniles by their begging responses to broadcasted adult hoot calls was possible as late as 24 wk post-banding. An option for longer-term monitoring of juvenile survivorship includes the temporary attachment of a radio transmitter prior to fledgling departure from the nest.

In this study, several methods of estimating exposure were applied. For select nests, fresh eggs were sampled shortly after identification of incubation activity, while other territories were monitored for productivity. Nestlings from these territories as well as nestlings from egg sampling territories (re-nesters or completed incubation of partial clutch sampling) were banded, 7 ml of blood collected, general health determined, and select morphological measurements taken. Prey remains (including pellets) were collected from active nests, base of the nest tree, and beneath nearby feeding perches for all nests in which fledgling productivity was monitored. Pellet and prey remains analyses identified 285 individual prey items.

In order to determine which type of egg sampling would have the least effect on territorial and site-wide productivity, fresh eggs were collected using two different sampling approaches. Either the entire clutch was collected to induce re-nesting or a single egg was left to induce continued incubation of the remaining egg(s). When the total clutch sampling approach was used, two of four pairs re-nested and produced three young. For nests in which the most recently laid eggs were left for continued incubation, three of seven pairs continued incubation. Two of the nests each produced one nestling and the third nest was destroyed by severe weather. In all, 24 eggs were sampled from 12 different territories.

Over the five-year study, the territories targeted for egg collection varied. Overall, the egg sampling effort targeted 24 territory-years in which 15 territory-years contained incubation activity. A territory-year is a level of effort term defined as any one territory monitored over one year. Thus, a single territory monitored over four years or four territories monitored over a single year both involve the same level of effort, 4 territory-years. The cumulative number of sampled eggs would have increased to 30 eggs had the entire clutch been collected for all territories targeted for fresh egg collections.

Conclusions resulting from each of the lines of evidence examined in this study were consistent between and among sites. Contaminant exposure based on both dietary- and tissue-based methodologies produced similar results of significantly different COC exposures for the downstream target vs. the upstream reference area. However, the results of the hazard assessments indicated that GHO populations residing in the floodplain were not at risk for effects induced by total PCBs or SDDT in contaminated soils. Maximum HQ values of <1.0 (diet exposures) and <1.5 (egg exposures) indicate that exposure of GHOs to the COCs in Kalamazoo River were below or near the threshold for effects (Strause, K.D., *et al.*, 2006a,b). Confidence in the risk conclusions was further strengthened by site-specific measurements of productivity, abundance and nestling growth and success. For each of these population parameters, target area owls were not significantly different from the upstream background areas, and were similar to those expected in a healthy environment. The mean rate of 1.0 fledgling per active nest observed at both locations is consistent with productivity measures for healthy mid-western GHO populations residing in upland habitats (Holt, J.B., 1996). Additionally, measures of site-use (abundance) indicate the target area populations at Trowbridge were near the carrying capacity for undisturbed GHO habitats (Houston, C.S., *et al.*, 1998). This consistency across each of the multiple lines of evidence for both measurement and assessment endpoints combined with the relative certainty of each measurement, the minimal impact on the receptor and environment, and the level of effort expended, highlights the utility of the GHO as a receptor in this and possibly other ERAs and natural resource damage assessment (NRDA) investigations.

Here we have provided an overview of the advantages of the GHO as a site-specific surrogate species for the determination of potential risk of contaminants in terrestrial ecosystems. We have given a short overview of a case history. The space available here was limited. For that reason, neither the methods nor the results could be fully described. Detailed methods in the form of standard operating procedures (SOPs), are available from the authors. In addition, detailed results of the assessments are published elsewhere (Strause, K.D., *et al.*, 2006a, b, c).

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Animal Use:

All aspects of the study that involved the use of animals were conducted in the most human way possible. Toward this end, all aspects of the study were conducted using standard operating procedures that had been approved (AUF #s 02/10-030-00; 03 /04-044-00; 08/03-105-00) by the MSU All University Committee on Animal Use and Care (AUAUC). All of the necessary state and federal approvals and permits obtained for the project (Michigan Department of Natural Resources (MDNR) Land Use Permit #A-02-09; MDNR Scientific Collecting Permit #SC1220; USFWS Migratory Bird Scientific Collection Permit #MB081272-1) were obtained are on file at the MSU ATL.

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Table 1. Sampling scope and blood plasma and egg summary. Description of sampling effort by year and location. Note that for 2000 - 2002 the Fort Custer and Trowbridge sampling areas were monitored for productivity thus only addled eggs were collected**.**

		Reference Sample Sites		Target Sample Sites	
Year		***Ceresco***	***Fort Custer***	***Trowbridge Impoundments***	***Allegan SGA***
2000	Active Nests		0	1	2
	Plasma		0	1	0
	Eggs		0	0	3
	Data Targeted[1]	NM	P, NP, RA	P, NP, RA	E
2001	Active Nests	1	1	2	1
	Plasma	0	1	4	0
	Eggs	1	0	0	2
	Data Targeted[1]	E	P, E, NP, RA	P, E, NP, RA	E
2002	Active Nests	2	0	4	2
	Plasma	1	0	3	2
	Eggs	2	0	1	5
	Data Targeted[1]	E, NP	P, E, NP, RA	P, E, NP, RA	E, NP
2003	Active Nests	1	1	2	1
	Plasma	1	0	1	2
	Eggs	1	1	3	0
	Data Targeted[1]	E, NP	E, NP	E, NP	E, NP
2004	Active Nests	0	0	2	2
	Plasma	0	0	0	0
	Eggs	0	0	3	2
	Data Targeted[1]	E, NP	E, NP	E, NP	E, NP

[1] NM=not monitored; P=productivity; E=egg sampling; NP=nestling plasma sampling; RA=relative abundance

Table 2. PCB concentrations in GHO dietary items sampled from proximal foraging areas. Waterfowl were not sampled based on sensitivity analysis.

Dietary items	N	***Trowbridge*** Mean total PCBs (± std dev) (mg/kg)[B]	N	***Fort Custer*** Mean total PCBs (± std dev) (mg/kg)[B]
Small mammal[A]	21	*0.13 ± 0.16	18	0.021 ± 0.042
House wren adult	6	*3.57 ± 2.30	5	0.09 ± 0.032
American robin	8	*1.14 ± 1.44	4	0.091 ± 0.65
Tree Swallow	5	*11.46 ± 11.90	2	1.49 ± 0.15
Shrew	17	*1.31 ± 0.94	16	0.009 ± 0.005
Muskrat	7	*0.07 ± 0.03	4	0.01 ± 0.01

[A]Includes; white-footed mouse, deer mouse, jumping mouse, meadow vole, red squirrel, and eastern chipmunk.
[B]On a wet-weight basis.
*Indicates a significant difference between sites at $p<0.05$.

Table 3. Relative abundance and reproductive productivity of GHOs. Abundance based on adult, juvenile and pair responses to great horned owl calls broadcast from predetermined locations throughout sampling areas.

	Ft. Custer	*Trowbridge*
Relative Abundance[1]	$N^2=24$	$N^2=22$
Adults	Mean Response Rate[3]	
Total[4]	1.31	2.76
Foraging[5]	0.85	1.64
Paired[6]	0.47	1.13
Juveniles	Response Frequency[7] n, (%)	
Fledgling	1 (4%)	8 (36%)
Productivity		
Active Nests	1	6
Fledglings	1	6
Fledglings/Nest	1	1

1. Derived from hoot call/response surveys completed at dawn and dusk.
2. N=number of complete surveys.
3. Mean response rate is averaged across N completed surveys.
4. Includes discrete responses from both individual and paired owls.
5. Includes responses from unpaired individuals only.
6. Includes responses from paired (male + female) owls only.
7. Response frequency of fledgling owls, n=number of surveys with at least one fledgling begging call response, (%)= (n) / number of surveys (N^2).

Figure 1. Map of sampling areas within Kalamazoo river floodplain. Superfund site extends 128 km from the city of Kalamazoo to its confluence with Lake Michigan. The sampling areas of Trowbridge and Allegan State Game Area lie 30 and 60km downstream of Kalamazoo while the reference sampling areas of Fort Custer and Ceresco lie similar distances upstream of the start of the Superfund site respectively.

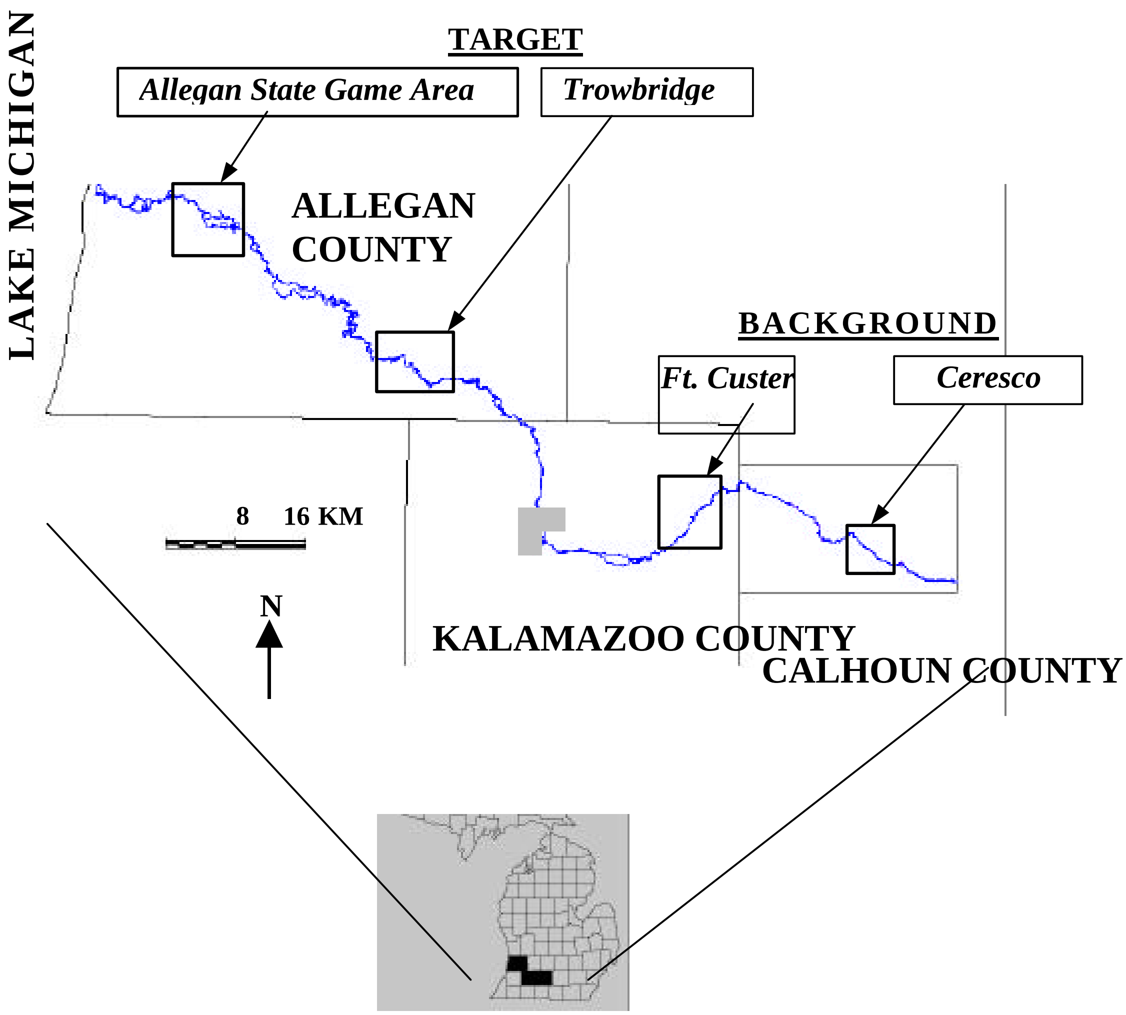

Figure 2. (Left) Artificial nesting platform (Right) Platform installation, Note; stainless steel adjustable pipe clamps are not camouflage painted for demonstration purposes.

Figure 4. Concentrations of PCBs and SDDTs in GHO tissues(egg). Median concentrations and associated one standard deviation of samples collected at four locations. Sampling locations presented from upstream to downstream (left to right) with the two reference sites upstream of point sources (Ceresco and Fort Custer), and two target sites downstream of point sources (Trowbridge and Allegan State Game Area).

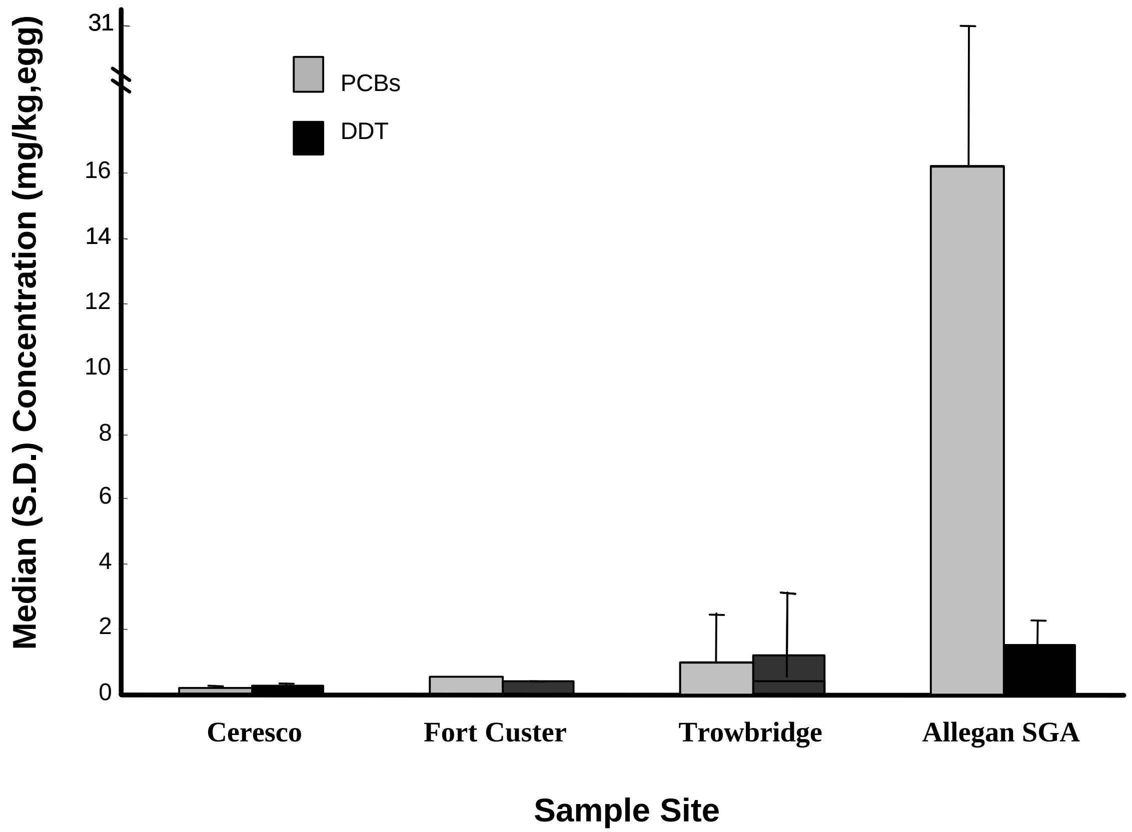

Figure 3. Dietary composition of GHO as determined by pellet and prey remains analysis. Data presented as percent frequency of occurrence from active nests within sampling area 2000-2002.